B. Construction of IgM expressing minilocus transgene, pIGM1

1. Isolation of J-μ constant region clones and construction of pJM1

A human placental genomic DNA library cloned into the phage vector λEMBL3/SP6/T7 (Clonetech Laboratories, Inc., Palo Alto, CA) was screened with the human heavy chain J region specific oligonucleotide:

oligo-1 5'- gga ctg tgt ccc tgt gtg atg ctt ttg atg tct ggg gcc aag -3'

and the phage clone λ1.3 isolated. A 6 kb HindIII/KpnI fragment from this clone, containing all six J segments as well as D segment DHQ52 and the heavy chain J-μ intronic enhancer, was isolated. The same library was screened with the human μ specific oligonucleotide:

oligo-2 5'- cac caa gtt gac ctg cct ggt cac aga cct gac cac cta tga -3'

and the phage clone λ2.1 isolated. A 10.5 kb HindIII/XhoI fragment, containing the μ switch region and all of the μ constant region exons, was isolated from this clone. These two fragments were ligated together with KpnI/XhoI digested pNNO3 to obtain the plasmid pJM1.

2. pJM2

A 4 kb XhoI fragment was isolated from phage clone λ2.1 that contains sequences immediately downstream of the sequences in pJM1, including the so called $\Sigma\mu$ element involved in δ-associated deleteon of the μ in certain IgD expressing B-cells (Yasui et al., Eur. J. Immunol. 19:1399 (1989), which is incorporated herein by reference). This fragment was treated with the Klenow fragment of DNA polymerase I and ligated to XhoI cut, Klenow treated, pJM1. The resulting plasmid, pJM2 (Fig. 23), had lost the internal XhoI site but retained the 3' XhoI site due to incomplete reaction by the Klenow enzyme. pJM2 contains the entire human J region, the heavy chain J-μ intronic enhancer, the μ switch region and all

of the μ constant region exons, as well as the two 0.4 kb direct repeats, $\sigma\mu$ and $\Sigma\mu$, involved in δ-associated deletion of the μ gene.

3. Isolation of D region clones and construction of pDH1

The following human D region specific oligonucleotide:

oligo-4 5'- tgg tat tac tat ggt tcg ggg agt tat tat aac cac agt gtc -3'

was used to screen the human placenta genomic library for D region clones. Phage clones λ4.1 and λ4.3 were isolated. A 5.5 kb XhoI fragment, that includes the D elements D_{K1}, D_{N1}, and D_{M2} (Ichihara et al., EMBO J. 7:4141 (1988)), was isolated from phage clone λ4.1. An adjacent upstream 5.2 kb XhoI fragment, that includes the D elements D_{LR1}, D_{XP1}, $D_{XP'1}$, and D_{A1}, was isolated from phage clone λ4.3. Each of these D region XhoI fragments were cloned into the SalI site of the plasmid vector pSP72 (Promega, Madison, WI) so as to destroy the XhoI site linking the two sequences. The upstream fragment was then excised with XhoI and SmaI, and the downstream fragment with EcoRV and XhoI. The resulting isolated fragments were ligated together with SalI digested pSP72 to give the plasmid pDH1. pDH1 contains a 10.6 kb insert that includes at least 7 D segments and can be excised with XhoI (5') and EcoRV (3').

4. pCOR1

The plasmid pJM2 was digested with Asp718 (an isoschizomer of KpnI) and the overhang filled in with the Klenow fragment of DNA polymerase I. The resulting DNA was then digested with ClaI and the insert isolated. This insert was ligated to the XhoI/EcoRV insert of pDH1 and XhoI/ClaI digested pGPe to generate pCOR1 (Fig. 24).

5. pVH251

A 10.3 kb genomic HindIII fragment containing the two human heavy chain variable region segments V_H251 and V_H105

(Humphries et al., Nature 331:446 (1988), which is incorporated herein by reference) was subcloned into pSP72 to give the plasmid pVH251.

6. pIGM1

The plasmid pCOR1 was partially digested with XhoI and the isolated XhoI/SalI insert of pVH251 cloned into the upstream XhoI site to generate the plasmid pIGM1 (Fig. 25). pIGM1 contains 2 functional human variable region segments, at least 8 human D segments all 6 human J_H segments, the human J-μ enhancer, the human $\sigma\mu$ element, the human μ switch region, all of the human μ coding exons, and the human $\Sigma\mu$ element, together with the rat heavy chain 3' enhancer, such that all of these sequence elements can be isolated on a single fragment, away from vector sequences, by digestion with NotI and microinjected into mouse embryo pronuclei to generate transgenic animals.

C. Construction of IgM and IgG expressing minilocus transgene, pHC1

1. Isolation of γ constant region clones

The following oligonucleotide, specific for human Ig g constant region genes:

oligo-29 5'- cag cag gtg cac acc caa tgc cca tga gcc cag aca ctg gac -3'

was used to screen the human genomic library. Phage clones 129.4 and λ29.5 were isolated. A 4 kb HindIII fragment of phage clone λ29.4, containing a γ switch region, was used to probe a human placenta genomic DNA library cloned into the phage vector lambda FIX™ II (Stratagene, La Jolla, CA). Phage clone λSg1.13 was isolated. To determine the subclass of the different γ clones, dideoxy sequencing reactions were carried out using subclones of each of the three phage clones as templates and the following oligonucleotide as a primer:

oligo-67 5'- tga gcc cag aca ctg gac -3'

Phage clones λ29.5 and λSγ1.13 were both determined to be of the γ1 subclass.

2. pγe1

A 7.8 kb HindIII fragment of phage clone λ29.5, containing the γ1 coding region was cloned into pUC18. The resulting plasmid, pLT1, was digested with XhoI, Klenow treated, and religated to destroy the internal XhoI site. The resulting clone, pLT1xk, was digested with HindIII and the insert isolated and cloned into pSP72 to generate the plasmid clone pLT1xks. Digestion of pLT1xks at a polylinker XhoI site and a human sequence derived BamHI site generates a 7.6 kb fragment containing the γ1 constant region coding exons. This 7.6 kb XhoI/BamHI fragment was cloned together with an adjacent downstream 4.5 kb BamHI fragment from phage clone λ29.5 into XhoI/BamHI digested pGPe to generate the plasmid clone pγe1. pγe1 contains all of the γ1 constant region coding exons, together with 5 kb of downstream sequences, linked to the rat heavy chain 3' enhancer.

3. pγe2

A 5.3 kb HindIII fragment containing the γ1 switch region and the first exon of the pre-switch sterile transcript (P. Sideras et al. (1989) International Immunol. 1, 631) was isolated from phage clone λSγ1.13 and cloned into pSP72 with the polylinker XhoI site adjacent to the 5' end of the insert, to generate the plasmid clone pSγ1s. The XhoI/SalI insert of pSγ1s was cloned into XhoI digested pγe1 to generate the plasmid clone pγe2 (Fig. 26). pγe2 contains all of the γ1 constant region coding exons, and the upstream switch region and sterile transcript exons, together with 5 kb of downstream sequences, linked to the rat heavy chain 3' enhancer. This clone contains a unique XhoI site at the 5' end of the insert. The entire insert, together with the XhoI site and the 3' rat enhancer can be excised from vector sequences by digestion with NotI.

4. pHC1

The plasmid pIGM1 was digested with XhoI and the 43 kb insert isolated and cloned into XhoI digested pge2 to generate the plasmid pHC1 (Fig. 25). pHC1 contains 2 functional human variable region segments, at least 8 human D segments all 6 human J_H segments, the human J-μ enhancer, the human $\sigma\mu$ element, the human μ switch region, all of the human μ coding exons, the human $\Sigma\mu$ element, and the human $\gamma 1$ constant region, including the associated switch region and sterile transcript associated exons, together with the rat heavy chain 3' enhancer, such that all of these sequence elements can be isolated on a single fragment, away from vector sequences, by digestion with NotI and microinjected into mouse embryo pronuclei to generate transgenic animals.

D. Construction of IgM and IgG expressing minilocus transgene, pHC2

1. Isolation of human heavy chain V region gene VH49.8

The human placental genomic DNA library lambda, FIX™ II, Stratagene, La Jolla, CA) was screened with the following human VH1 family specific oligonucleotide:

oligo-49 5'- gtt aaa gag gat ttt att cac ccc tgt gtc ctc tcc aca ggt gtc -3'

Phage clone λ49.8 was isolated and a 6.1 kb XbaI fragment containing the variable segment VH49.8 subcloned into pNNO3 (such that the polylinker ClaI site is downstream of VH49.8 and the polylinker XhoI site is upstream) to generate the plasmid pVH49.8. An 800 bp region of this insert was sequenced, and VH49.8 found to have an open reading frame and intact splicing and recombination signals, thus indicating that the gene is functional (Table 2).